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Microbial Degradation of Paintings

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"*. . .l'alliance possible et désiderable de la Science et de l'Art. . .*,"
Louis Pasteur, when he was nominated to the first chair in physical chemistry at the Ecole des Beaux Arts in Paris.

INTRODUCTION

In 1940 four young men discovered the Lascaux Cave in the Dordogne region of France. The cave contained an impressive display of prehistoric art: the main cavern and several galleries connected to it were decorated with engraved, drawn, and painted figures of animals. The approximately 600 paintings, done with mineral pigments mixed with animal fat in various shades of yellow, red, brown, and black, were dated to the late Aurignacian period (15,000 to 13,000 B.C.). With few exceptions, the paintings, some as long as 5 m, represented different animals (some imaginary), and their quality was such that the cave was designated by some the Sistine Chapel of the Paleolithic. In 1948 Lascaux Cave was opened to visitors, but in 1963 it was closed indefinitely to the public. Closing was imposed after the discovery of a green patina (from which comes the term *maladie verte*, or green disease) covering the painted portions (34). Quite unexpectedly, although other algae together with cyanobacteria, bacteria, and fungi were isolated in different parts of the cave, the green patina was composed exclusively of the unicellular alga *Bracteacoccus minor* (order *Chlorococcales*). The influx of workers and visitors brought into the cave considerable amounts of soil and of the organic compounds present in people's breath and sweat and increased the concentration of carbon dioxide to almost pathological levels. The lighting system, installed in the cave and operating almost continuously, created the conditions for a massive growth of photosynthetic organisms. Extensive analysis of the composition of, and the variations in, the microbial population of the painted areas as well as of the unpainted rocks and the surrounding environment led to the conclusion that the population of *Bracteacoccus minor*, responsible for the *maladie verte*, also increased when the cave was closed to the public and kept in continuous darkness for long periods. Indeed, after 3 months of total darkness and closure to the public, algal proliferation on painted areas was found to have increased by 1 order of magnitude (35). Thus, it was concluded that the alga could grow even under heterotrophic conditions by utilizing the organic molecules brought in the cave by visitors or resulting from the degradation of biological residues. It was postulated that, before discovery and opening of the cave, the community of heterotrophic microorganisms, bacteria and fungi, present in the cave had mineralized all organic molecules present, so that heterotrophic growth of the alga was prevented, as was autotrophic growth as a result of the absence of light.

The Lascaux Cave is perhaps the most emblematic example of the damage that microorganisms may cause to art work and should settle once and forever the arguments about the possible role of microorganisms in the degradation of our cultural heritage. The conditions that led to the microbial bloom on the Lascaux Cave paintings probably represent an extreme case, but it may be argued convincingly that even less harsh environmental stresses than those that occurred in the less than 20 years since the opening of the Lascaux Cave may cause irreversible aesthetic and structural damage to almost any type of art work.

This minireview focuses on the colonization of art works by microorganisms and its effects. Its scope will be limited to paintings, both on canvas and panel, as well as on walls. Thus, other art works, such as those in stone, wood, paper, and masonry, as well as those in more esoteric materials, such as leather, parchment, glass, and metal, will not be considered. For a more comprehensive treatment of the role of microorganisms in the degradation of our cultural heritage, the reader should refer to the reviews already published (2, 6, 12, 13, 20, 29, 30, 36, 46, 55, 56). The treatment of the subject will not be exhaustive but will focus on aspects that, in the writer's opinion, appear to be most interesting. At the end, a few ideas on how, again in the writer's opinion, the research in this field might proceed will be expressed.

THE SUBSTRATE

Paintings, whether easel or mural, contain a wide range of organic and inorganic constituents and provide different ecological niches that may be exploited by a large variety of microbial species. Many of the components of paintings are biodegradable, and so are the additives (glues, emulsifiers, thickeners, etc.) that facilitate drawing or application of paint layers or enhance the aesthetic quality of the finished product.

In easel paintings, the support material (the cellulose of paper, canvas, and wood and the proteins of parchment, silk, and wool) may be easily degraded by microorganisms, as may the materials (animal or plant glues) used to "size" the support and to prepare a ground layer. Paintings on paper or silk are laid, in general, directly on the support, since a ground or underlay is lacking, but the pigments are kept in emulsion with organic binders. Thus, besides the organic nature of the support, easel paintings contain organic molecules that many microorganisms may utilize for growth, such as sugars, gums, and other polysaccharides, proteins, linseed and other oils, waxes, etc., but also less chemically defined mixtures of biomolecules such as egg yolk, bile, and even urine. (A list, certainly not exhaustive, of the organic components that may be present on paintings can be found in references 15 and 55).

Mural paintings rely on techniques and materials differing from those utilized in easel paintings. Essentially, pigments are suspended in water or oil, often in the presence of binders such as casein and milk, and applied on the damp lime plaster. The calcium carbonate formed on contact with air consolidates the pigments. Thus, by and large, frescoes contain mainly inorganic components and the microbial flora that colonize these substrates may, at least in the first steps, differ from that

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present on easel paintings. For both types of paintings the spectrum of compounds that may be present is further increased by those that are added at later times during retouching, restoration, or relining or when a fresco is detached and transferred to a canvas or a board. In one case at least, extensive fungal colonization was reported even with frescoes that, after cleaning and consolidation, were removed from walls and transferred to a fiberglass support (42). Finally, dirt, soot, and other environmental contaminants, accumulating on the painted surface, may represent another not insignificant source of nutrients.

Given the wide range of organic and inorganic molecules that are present in both types of paintings, many different types of microorganisms may grow on such substrates provided that favorable environmental conditions (humidity, temperature, light, and, to a lesser extent, pH) are met. It sounds almost tautological to state that, besides the chemical composition of an art work, the environment conditions the development of a microbial flora, as it is quite obvious that a specific microbial flora will develop, for instance, on a fresco on the facade of a church where it receives a considerable amount of light and a different flora will develop on a similar fresco inside the same building in which light is very reduced. Likewise, if temperature, moisture, and light are not controlled, the microbial communities of two paintings produced with exactly the same materials will differ considerably if one painting is kept in the northern latitudes and the other is kept in the tropics. It may be added that high levels of humidity, temperature, and light, as may be found, for instance, in warmer climates, may shorten the, one could say, life span of a painting by exacerbating the damages caused by air pollution, biological attack, and natural aging.

Growth of microorganisms on paintings may cause aesthetic and structural damage. As aesthetic damage one must consider pigment discoloration, stains, and formation of a biofilm on the painted surface, whereas as structural damage one must consider cracking and disintegration of paint layers, formation of paint blisters, and degradation of support polymers or of glues and binders resulting in detachment of the paint layer from the support. Of course, the two types of damage are strongly linked, and in the long run, structural damage profoundly affects the aesthetic quality of a painting. Conversely, aesthetic damage may precede serious injuries to the materials. For instance, in fungal colonization of mural paintings, Saiz-Jimenez and Samson (47) have shown that, at the beginning, growth of fungi on a mural's surface caused only aesthetic damage since there was little or no alteration of the painted surface. Later on, fungal growth in depth occurred. Hyphae penetrated the painted layer, degrading some of its components (especially glues and binders), which resulted in a decrease in the cohesion of the painted layers, thus giving rise to exfoliations, cracking, and loss of the paint. To these damages one should add those inflicted by metabolites, often acidic in nature, and by extracellular enzymes excreted by microorganisms. These compounds may modify the colors as well as the stability of the painted layer and of the substrate.

Similarly, cyanobacteria and algae growing on paintings exposed to light, such as frescoes on the facades of buildings, may cause considerable damage. Besides the aesthetic damage caused by a green, black, brown, or yellow algal patina covering the painted portions, these organisms may cause weathering of the surface layers, accelerating detachment of portions of the painted layer as well as the underlying plaster (40). The presence in a number of Italian frescoes of species of nitrogen-fixing *Nostoc* indicates that cyanobacteria may colonize frescoes in which combined nitrogen may be absent (58). Indeed, in this investigation, determination of acetylene reduction in situ demonstrated that nitrogen fixation occurred, albeit at a reduced rate, in the microbial biofilm covering the frescoes. In addition, cyanobacteria and algae can provide an important source of organic material on which heterotrophic bacteria and fungi may thrive, thus causing further aesthetic and structural damage to the paintings. Finally, cyanobacteria and algae may colonize the mortar, bricks, or stone supporting frescoes. Indeed, these organisms have been reported to contribute to the weathering process of masonry (31).

THE FLORA

With a few exceptions, characterization of the microbial flora present on frescoes or easel paintings has been limited to selected groups of microorganisms rather than to all types of microorganisms that might be present on a given substrate. Thus, in general, surveys have often been limited to fungi (1, 8, 10, 14, 17, 22–26, 37, 47, 50, 57), bacteria (7, 18, 32, 33, 44, 45), or cyanobacteria and eukaryotic algae (9, 16, 21, 40, 58). In a few cases, more comprehensive analyses aiming to determine all, or the majority of, the biota present on a painting have been reported (27, 41). This comprehensive data may provide the foundation for ascertaining the existence of associations or successions among the components of a microbial flora. Recently, a method of identifying microorganisms by sequencing a portion of the DNA coding for the 16S rRNA has been used with cultures of bacteria isolated from frescoes (7, 44) and even with DNA samples extracted directly from a fresco (44, 45). This technique, extensively employed in macromolecular ecology to identify, without culturing, members of microbial communities, will certainly lengthen the list of microorganisms present on any given substrate by permitting, for instance, the identification of species that are present at very low cell concentrations and of those that cannot be cultured in the laboratory. However, it will not determine if the DNA derives from living or dead microorganisms and, more importantly, it will not allow us to distinguish between microorganisms responsible for the observed damage (one could call them the parasites) and those that do not contribute to it (the saprophytes). Similar limitations will greatly reduce the usefulness of other molecular biological techniques, such as fluorescence in situ hybridization, that permit identification of microorganisms without their isolation and culture.

Perusal of the lists of taxa isolated shows that the most common soil inhabitants, both fungi (species of *Penicillium*, *Aspergillus*, *Cladosporium*, *Chaetomium*, and *Alternaria*) and bacteria (species of *Pseudomonas*, *Arthrobacter*, and *Streptomyces*), are present in many of the samples analyzed. However, wide quantitative variations are evident. For instance, from a fresco in St. Damian's Monastery in Assisi, Italy, more than 33 different species of fungi belonging to at least 17 genera were isolated (approximately 25% of all isolates were not identified) (22). On the other hand, from a mural in Canterbury Cathedral only one fungal species, *Beauveria alba* (*Engyodontium album*), was repeatedly isolated (26) and, similarly, on damaged frescoes in an Italian church only one species of *Cladosporium* was found (37).

Gettens and coworkers were among the first to point out, in 1941, that paintings could be "defaced or destroyed by the growth of those small, parasitical plants commonly called 'mold' or 'mildew'" (15). Further, in laboratory experiments, they demonstrated that treatment with fungicides could arrest or prevent microbe-induced damage to paintings. About 20 years later, Tonolo and Giacobini (59) confirmed that microorganisms could damage works of art by providing examples of frescoes disfigured by growth of eukaryotic algae (members of *Chlorophyceae*), bacteria (*Sarcina lutea* or *Streptomyces* spp.), or fungi (species of *Penicillium*, *Aspergillus*, *Cephalosporium*, and some *Dematiaceae*). The authors reported that these organisms could cause changes to the paintings' surfaces through staining, discoloration, or formation of patinas and efflorescence. In addition, they showed that many such organisms, especially the fungi, could grow between the paint layers and the ground, causing a swelling of the paint film that could lead to detachment of portions of the painted layer and disaggregation of the underlying ground. This in turn could promote separation of the painted surface from the ground or of the ground from the masonry on which the fresco was laid.

After these pioneering papers, Gargani (14) and Tiano and Gargani (57) published a detailed investigation of the microbial floras of art works, mostly frescoes. Their work was greatly stimulated by the finding that, after the flooding of Florence in November 1966, a great number of paintings, both mural and easel, were severely damaged and that the damage could be at least in part associated with the growth of microorganisms. Using a technique of dermatologic mycology, they determined that direct microscopic examination of the microbial structures adhering to transparent cellulose tape pressed on the painted surface revealed the presence of fungal elements, such as hyphae, typical of most filamentous fungi. However, species identification and determination of the microbial load were possible only when cultures on different media were made with small fragments of the painted surface or cotton swabs brushed on such a surface. The analyses were essentially limited to the fungal population and demonstrated that clear differences existed between the numbers of species isolated from art works and those isolated from the environment in which the art work was located. For instance, from the surface of a fresco by Beato Angelico in St. Mark's Convent in Florence, Italy, 17 different species of hyphomycetes encompassing 10 genera were isolated whereas from the environment 9 species (six genera) were isolated. These data could be taken as an indication of the presence of a fungal flora specifically developing on the painting and differing, at least in part, from that present in the environment. However, sampling at different intervals (months) revealed significant differences in the compositions of the flora of the painted surface whereas there was little variation from sampling to sampling in the flora of the environment. Similar wide variations in the species isolated from different periods were reported in the analyses of the microbial, essentially fungal, flora present on wall paintings in the Buddhist shrines of Ajanta in India (1). Of 40 different species of fungi isolated from the wall paintings on three different visits, only 11 species were always present and more than 50% were isolated only once.

Two 15th-century murals in the Ognissanti church in Florence, restored in 1969 after the flood of 1966, were cleaned and treated with nystatin in the late 1970s but, in 1985, showed the appearance of greenish-brown–to–black spots on the painted surface (49). Isolation of fungal species from such areas demonstrated the presence of 15 different species from the samples taken from Botticelli's fresco and a similar number (13) from that by Ghirlandaio. However, striking differences in the types of species were evident: the most abundant fungi on the fresco by Botticelli were two species of *Penicillium* and *Cladosporium cladosporiodes*, whereas the most common fungi in the fresco by Ghirlandaio were *Aspergillus versicolor* and *Cladosporium sphaerospermum*. Even more striking was the finding that the two penicillia most abundant on the Botticelli fresco were undetected on the fresco by Ghirlandaio, as was the case with *Aspergillus versicolor*, which accounted for 74% of the isolates from Ghirlandaio's fresco but was not isolated from Botticelli's fresco. Such differences in two frescoes painted at the same time (1480) in the same building, presumably with similar or identical materials, and restored and cleaned at the same time appear rather striking. In laboratory experiments, 19 species of the fungi isolated from the two frescoes were tested for the capacity to grow on the materials used for restoration (calcium caseinate, animal glue, and masonite, used as a support panel). Although qualitative differences were observed, essentially all the fungal species isolated from both frescoes grew quite well on calcium caseinate, to a lesser extent on masonite, and to an even lesser extent on animal glue. The only exception was provided by the two species of *Cladosporium*, which, although being among the most frequent isolates from the two frescoes, did not grow well on any of these materials. In the opinion of the investigators, this genus is one of the most commonly isolated from frescoes because it is resistant to variations in external factors (temperature, humidity, etc.). However, as the two tested species of *Cladosporium* did not grow on casein, masonite, or animal glue, the investigators assumed that this genus did not contribute significantly to the degradation of paintings. This assumption is in contrast to the opinion of other scientists who consider *Cladosporium* one of the major biological agents, if not the most significant agent, responsible for fresco degradation (2, 19, 37, 47). In conclusion, the differences observed in the fungal colonizations of the two frescoes are not easily explained. Assuming that no great differences exist in the materials used when the two frescoes were painted (but this cannot be proved), the only possible explanation is that the locations of the two frescoes in the church are such that they affect differentially the fungal colonizations of the two murals. One can argue that the positions of the frescoes relative to openings (windows or doors), sources of moisture and heat, and other factors may be responsible for the differences in the fungal colonizations. In an extensive investigation of the fungal colonizations of frescoes in eight different Moldavian monasteries, Ionita (25) isolated 26 different species of fungi from stains appearing on the frescoes, from areas of efflorescence, and from zones in which the painted layer was fissured and portions were breaking away from the support. No apparent recognizable pattern in the fungal distribution could be observed. For instance, from three areas with stains of the same color, present on the same portion of a fresco, different fungi were isolated. In addition, the same fungal species was isolated from spots of different colors as well as from fissured fragments of the frescoes that were apparently not stained. Further, *Aspergillus niger*, one of the most ubiquitous fungal contaminants, was isolated in only one case.

Such variations in the fungal floras present in samples taken at different times, or in frescoes of the same age and in the same location, were often observed and do not allow us to establish conclusively that the fungi present on a painted surface, even when they are absent from the environment, are responsible for the damage observed on the paintings. Further, no attempt has been made to identify the species responsible for the damage, both aesthetic and structural, and the species that are just saprophytes living on the painted surface may be growing at the expense of other microorganisms colonizing the frescoes. However, the idea that fungi may be the primary microbiological agents responsible for degradation of art works is so entrenched that often antibacterial agents are added routinely to the media used for the isolation of the microbial contaminants presumed responsible for the degradation of art works (22, 26).

With frescoes located underground, such as those in crypts, tombs, and grottoes, it has been reported that the predominant species and, possibly, the first colonizers are members of the order *Actinomycetales*, most of which are in the genus *Streptomyces* and a few of which are in the genus *Nocardia* (18). Over 200 strains of actinomycetes were isolated from 13 frescoes in different Italian hypogean sites. In some of these, cell concentrations reached up to 1 million cells/gram of sample. Of the 200 isolates, 46 were identified as members of 19 different species of *Streptomyces* and 5 were identified as members of the genus *Nocardia*. According to the researchers, colonization by actinomycetes begins as soon as the sites are opened and the frescoes are excavated, becoming quite evident only 2 months after excavation and exposure to air. In a short period, other microorganisms (bacteria, fungi, and algae) become associated with the predominant population of actinomycetes. When the hypogean rooms of the Domus Aurea in Rome were opened to visitors in 1951, very rapidly green crusts appeared on the frescoes in lighted areas; their development was so rapid that, in 1981, illumination had to be discontinued (21). A study of the microbial community composing such crusts showed a predominance of cyanobacteria (two species of *Lyngbya*, accompanied by unidentified bacteria) and chlorophytes (species of *Chlorella*, *Pseudococcomyxa*, and *Pseudopleurococcus*) (4). The composition of the algal population associated with the damage was studied for 4 years. During this period, the two species of *Lyngbya* were by far the predominant ones. The chlorophytes *Pseudococcomyxa simples* and *Pseudopleurococcus printzii* were always present but at much lower cellular concentrations. These findings were confirmed in laboratory experiments in which samples of the microbial mats from the frescoes were grown under fluorescent or incandescent light at two different light intensities (5). In these experiments too the two *Lyngbya* species appeared to be the predominant ones. According to the investigators, the presence of thick sheaths of these cyanobacteria not only favored their adhesion to the painted surface but provided also the substrates for the establishment of a population of heterotrophic bacteria (3).

In conclusion, although an impressive number of publications report that from damaged paintings it is possible to isolate a wide range of microorganisms, with very few exceptions no attempts have been made to distinguish between microorganisms responsible for the deterioration and those that play no role, direct or indirect, in the process leading to a painting's defacement.

MECHANISM OF AGGRESSION AND MICROBIAL SUCCESSION

Presenting a unified scheme for determining the mechanism of microbial damage of painted surfaces is rather difficult. Such difficulty resides in the fact that the chemical compositions of paintings vary considerably, and at times, they are even impossible to ascertain. Although historical records and, more significantly, chemical analyses may indicate with sufficient accuracy the pigments that have been used in older art works, it is less easy to determine which components were used for sizing the ground, emulsifying the pigments, protecting the finished painted surfaces, etc. As already mentioned, another difficulty lies in the fact that most of the published reports are essentially catalogues of the microorganisms isolated from painted surfaces, especially from the areas in which visual inspection has revealed aesthetic damage due to changes in the colors of paints and appearance of stains, variations in the structure of the painted layer, etc. Further, as already noticed, quite often the lists of microorganisms isolated from a damaged painting are limited to one group of microorganisms (fungi, bacteria, or algae) and rarely include all the microorganisms present.

Nevertheless, in a few cases attempts have been made to present a more comprehensive analysis of the different microbial groups present, to unravel the chemical modifications brought about by the microbial colonization, and to determine the succession of the microbial colonizers. For instance, Saiz-Jimenez and Samson (47) have analyzed the microbial flora of a large fresco painted in the late 1920s in an old Spanish monastery. Two types of aesthetic damage were observed, white efflorescence and green-to-black stains. From both types of alterations *Cladosporium sphaerospermum* was the fungus most frequently isolated (approximately 75 to 88% of all isolates from the two types of lesions), followed by *Engyodontium album* (slightly more than 10% of all isolates from both efflorescent and stained areas). However, the fungi were considered secondary colonizers of the fresco. The first microorganisms colonizing the fresco were supposed to be sulfur-cycling bacteria (48), well known to play an important role in stone and masonry deterioration (12). The decay of the fresco was thought to have begun around the 1970s, coincident with the establishment in the vicinity of the monastery of a series of industrial plants that emitted into the atmosphere considerable amounts of pollutants, especially sulfur dioxide. The sulfuric acid produced from sulfur dioxide dissolved the calcium carbonate of the fresco, leading, eventually, to the production of a precipitate of dihydrous calcium sulfate (gypsum). Gypsum deposition resulted in the formation of white crystal aggregates responsible for the efflorescence observed on the fresco. Microbiological analyses showed that the efflorescence contained up to 65,000 sulfur-oxidizing and 200 sulfur-reducing bacteria per gram. In the investigator's view, the sulfur-utilizing bacteria were the first colonizers of the fresco. Death and lysis of these bacteria provided the organic substrates necessary for the growth of heterotrophic bacteria and fungi. Growth of the latter was considered responsible for the colored stains present on the fresco's surface as well as the mechanical damage observed, such as the detachment of portions of the painted layer. Thus, environmental pollutants, especially sulfur dioxide and related compounds, caused direct damage to the fresco but also provided the substrates that promoted growth of aerobic and anaerobic sulfate-cycling bacteria. These, in turn, supplied the organic nutrients that allowed the establishment of a community of scavenger bacteria and fungi that further contributed to the degradation of the fresco.

A somewhat similar sequence of events was postulated by Karpovich-Tate and Rebrikova to occur on frescoes and masonry in a Russian cathedral (27). According to these researchers, even in the presence of organic substrates such as components of fresco and plaster, the first colonizers were the autotrophic, nitrifying bacteria found on many different types of stone and masonry and considered responsible for the biologically induced corrosion of stone and other building materials (11). These bacteria oxidized to nitrate the ammonia present in the atmosphere and thus promoted growth of heterotrophic microorganisms (bacteria as well as fungi that were present in concentrations up to 10^6 cells/g of material) that also utilized the cellular components of the first colonizers. According to the researchers, support for this conclusion was given by the finding that most of the heterotrophs that were present on frescoes were capable of hydrolyzing bacterial and yeast cell walls. A somewhat similar analysis of the bacteria present on frescoes in northern Moldavia monasteries gave different results. Over 90 bacterial strains were isolated, all of which were heterotrophs and most of which were in the genera *Bacillus*, *Arthrobacter*, *Micrococcus*, *Sarcina*, and *Pseudomonas* (32). The presence of bacteria was constantly demonstrated in the samples collected from portions of the fresco disfigured by a whitish, powdery layer, whereas the absence of bacteria was demonstrated in samples from apparently undamaged portions of the fresco. In a courageous attempt to verify Koch's postulates, control experiments demonstrated that when pure cultures of many of these bacteria were transferred to sterile cotton wool wads and these were applied to and kept on undamaged portions of the same fresco for 3 to 4 weeks, almost half of the 40 isolates tested produced stains similar to those observed in the damaged portions. From the artificially produced areas of staining the researchers reisolated the bacterial species used for inoculation. Bacteria, especially of the genus *Arthrobacter*, were reported to be among the first colonizers of murals in a medieval church in Rostov, Russia (41), and to be responsible for oxidation of the lead present in pigments, resulting in the production of brown-black spots of lead oxides. Indeed, when samples taken from the damaged areas of the murals or bacteria isolated from such samples were incubated in mineral media in the presence of lead-containing pigments such as white lead, lead ocher, or red lead, good microbial growth together with the formation of a brown precipitate composed of lead dioxide was observed. The fact that no brown precipitate was formed in uninoculated media or in those inoculated with an unidentified fungus isolated from the same area of the fresco gave strong, presumptive evidence that the bacteria present in the damaged fresco were responsible for the oxidation of divalent lead to tetravalent lead oxide and hence to the appearance of dark-brown–to–black spots on areas in which lead-containing pigments were used. In addition, other laboratory experiments indicated that black spots of lead sulfide could be produced on the frescoes from the reaction between the lead oxide of pigments and the hydrogen sulfide produced by other bacterial species present in the samples.

In conclusion, the few reports in which the mechanism of microbial colonization of frescoes has been investigated indicate that bacteria may be the first colonizers. However, the majority of reports are limited to analyses of the fungal flora isolated from the substrates and make no attempt to establish whether these microorganisms are the first to colonize the substrates.

With easel paintings, experiments performed on wood panels coated with a white acrylic latex and exposed to soil in an environmental cabinet or in the field led to the isolation of members of 7 bacterial genera and 15 fungal genera, with no great difference between the numbers of genera isolated in the laboratory (20 isolated) and in the field samples (23 isolated) (38). The time course (over 2 weeks) of the colonization by the different genera showed that some organisms, termed transient species (*Acremonium*, *Penicillium*, and *Helmintosporium* spp.) were present only during certain periods but that not only were other organisms, termed permanent species (*Alternaria* and *Pseudomonas* spp.) present in all samples but also their numbers often increased throughout the period of exposure. Members of the genera *Alcaligenes*, *Bacillus*, *Flavobacterium*, and *Pseudomonas* represented the most frequent bacterial species present at all times. Whereas the population of most bacterial species remained constant or increased only slightly during the duration of the experiments, that of *Pseudomonas* increased linearly with the time of incubation (during 12 weeks of incubation, the number of colonies of *Pseudomonas* spp. per square centimeter increased by more than 1 order of magnitude). With fungi, only colony numbers of *Aureobasidium* (*Pullularia*) *pullulans*, considered by some the main biological agent of paint deterioration (28, 43), increased steadily with the time of incubation so that, after 12 weeks, this species was essentially the only fungal species present on the panels. Such results confirmed those of an earlier report on the succession of fungi on this type of paint, namely, that initially species of *Aspergillus*, followed by species of *Alternaria*, and, eventually, *Aureobasidium pullulans* were found. The last represented 80% of the climax community, the remaining 20% being represented by *Alternaria* spp. (60). The possibility that *Aureobasidium pullulans* grew at the expense of the polysaccharides of the *Pseudomonas* capsules and the other bacterial species colonizing the panels was investigated (39). Although dead bacterial cells adhering to the paint layer did stimulate growth of the fungus, further experiments provided evidence that bacterial colonization of the painted surface had chemically modified some of the components of the paint, rendering them utilizable by the fungus (38). Indeed, a previous report showed that *Aureobasidium pullulans* was unable to utilize hydroxyethylcellulose, a component of the paint, for growth but that it utilized this compound pretreated with cells of *Pseudomonas* or even with a cellulase produced by the bacterium (51).

Somewhat different conclusions were reached in our investigations with samples of painted canvases (mock paintings) prepared with traditional materials by following the standard recipes used for paintings (52). Essentially, mock paintings consisted of a linen canvas, sized with animal glue in water and with a ground of chalk and animal glue. A paint film of lead white in linseed oil was laid on the smoothed-out ground. The main soil microorganisms, fungi and bacteria, growing on the mock painting were identified. *Bacillus pumilus* was the bacterial species present at the highest cell concentration, by far, and *Aspergillus niger* and *Penicillium chrysogenum* were the fungal species present at the highest cell concentrations. Reconstruction experiments showed that pure cultures of the main bacterial species, including *Bacillus pumilus*, essentially did not grow when they were incubated with mock paintings. In this type of experiment only the viable counts of the fungi *Aspergillus niger* and *Penicillium chrysogenum* increased in the first period of incubation. However, the presence of *Aspergillus niger* stimulated growth and survival on mock paintings of *Bacillus pumilus* and the stimulatory effect of the fungus was abolished by the addition of cycloheximide, an inhibitor of protein synthesis and growth in eukaryotes but not in prokaryotes. These findings, indicating that growing fungal cells are necessary to promote growth and survival of *Bacillus pumilus*, could be explained by the fact that *Aspergillus niger* was found to possess cellulolytic and proteolytic activities, activities that were not identified in *Bacillus pumilus* and the other bacteria, all of which were gram positive, isolated from mock paintings exposed to soil. Thus, it was postulated that the fungus stimulated growth and survival of the bacteria by supplying the latter with the products of the hydrolysis of macromolecules, such as cellulose and proteins, present on the paintings. This conclusion was strengthened by the finding that the most abundant bacterial species, mostly gram-negative organisms, isolated from a severely degraded 16th century fresco that had been transferred in the 19th century to a canvas support hydrolyzed cellulose and casein, grew to a certain extent, and survived for a longer period of time on mock paintings than did the bacteria isolated from soil (53). Unlike *Bacillus pumilus*, growth and survival on mock paintings of the bacteria isolated from the fresco were not stimulated by the presence of *Aspergillus niger*. The differences between our data and those of O'Neil's and Schmitt's could be easily explained by the differences in the materials used (acrylic paint on wood in one case, oil paint on cloth in the other) and indicate that the succession of the different microbial taxa colonizing works of art depends also on the chemical nature of the substrate.

Indeed, work under way in my laboratory has demonstrated the existence of differences in the microbial colonizations of mock paintings when different pigment binders (oil or distemper) were used or when the same type of painting was relined with different glues (unpublished data).

That the chemical nature of the substrate conditions the capacity of microorganisms to colonize different art works was further demonstrated by the finding that silk (composed of the proteins fibroin and sericin but often of fibroin only) is easily colonized and degraded by bacteria (especially species of *Pseudomonas* and *Arthrobacter*) but that it is hardly attacked by fungi (54). However, if the textile was artificially aged in the laboratory by exposure to the light of a xenon lamp or to heat, treatments that result in a chemical modification of the protein, then it became susceptible also to fungal attack (unpublished data).

Thus, one should take into consideration how the microbial flora colonizing an art work varies according to the chemical composition of such a work. Further, the biochemical reactions catalyzed by the different microbial species may vary with the different makeup of the substrate and also when external factors, including age, alter the chemical structures of some of the components of the substrate. The number of variables to be taken into consideration becomes almost unlimited, presenting a difficult but not unsurmountable challenge, since reliable information can be gathered in laboratory experiments performed with standardized models. When the microbiologist is confronted with a request to investigate the presence (and role) of microorganisms on a defaced art work, he or she is called to do so with a substrate on which, quite often, microbial colonization has taken place for years. The microbial flora that he or she will find is probably the result of successive colonizations by different groups of microorganisms. Such variations are the result of modifications of the chemical composition of the substrate, to which the microorganisms themselves may have contributed in part. Thus, the investigator will have only a snapshot of the state of the artifact at that precise moment and not a time-elapsed picture of the development of the microbial communities that may have existed during the life span of the art work. In addition, he or she will be called to give an answer in a short period and to provide in great haste the information necessary for corrective interventions. A microbiologist should be asked to characterize the microbial flora present on a work of art when it appears to be still in its pristine condition and well before any alteration becomes evident. By determining which microorganisms are present at time zero, he or she will be able to make a reasonable assumption about how the microbial colonization will develop. In this way, the microbiologist will be able to suggest the nature and the mode of treatment that will stop microbial colonization before damage becomes visible and irreversible.

It seems to me that the time is now ripe to acknowledge that studies of the microbial colonization of art works should go beyond the descriptive stage, that is, cataloguing which organisms are found on which substrate. It is undeniable that this type of information is important to establish which organisms, or which types of organisms (bacteria, algae, fungi, etc.), colonize a given art work, since this information is necessary for any disinfestation treatment. However, I think that we are now in the position to begin to study and understand the mechanisms underlying the microbiological attack. In other words, we should try to set up standardized laboratory models using the most common types of support as well as the most commonly employed ingredients. These models will allow us to establish, under controlled conditions, which species colonize a given substrate, how the microbial flora will change on changing of the substrates (supports, pigments, binders, glues, etc.) that make up an art work, how the substrate is modified by the microbial colonization, and how these modifications lead to the establishment of different microbial communities. Similarly, one should try to evaluate in the laboratory how the microbial population varies when the environmental conditions change (a painting on the exterior of a building will undergo colonization by microorganisms different from those colonizing a similar painting located inside the same building). Finally, one must evaluate how aging, which may be simulated in the laboratory, may bring about variations in the chemical structures of many components of works of art (from the support polymers to the different binders and glues) and how these chemical variations may influence the colonization by different microbial taxa. From such research it will be possible to learn how to monitor and evaluate the onset and the rate of microbial colonization and the changes in the microbial population as a function of the substrate composition and environmental conditions and, eventually, how to proceed for disinfestation.

Finally, these data will be useful in indicating the most suitable materials to be used, including those for restoration and relining. We expect the life spans of works of art to be on the order of centuries if not millennia. It is inconceivable that we will find compounds that will ensure protection from microbial attack for periods of such lengths. If, for any reason, control of humidity, temperature, and light, as occurs in museums, is not possible, then protection of objects of artistic or historical interest rests only on the intrinsic components of such objects that can render them refractory to microbial colonization.

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